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Lyophilic matrix method for dissolution and release studies of nanoscale particles

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Highlights:

• The lyophilic matrix (LM) method for dissolution and release studies of powders, nanoscale particles, and particulate systems is introduced.

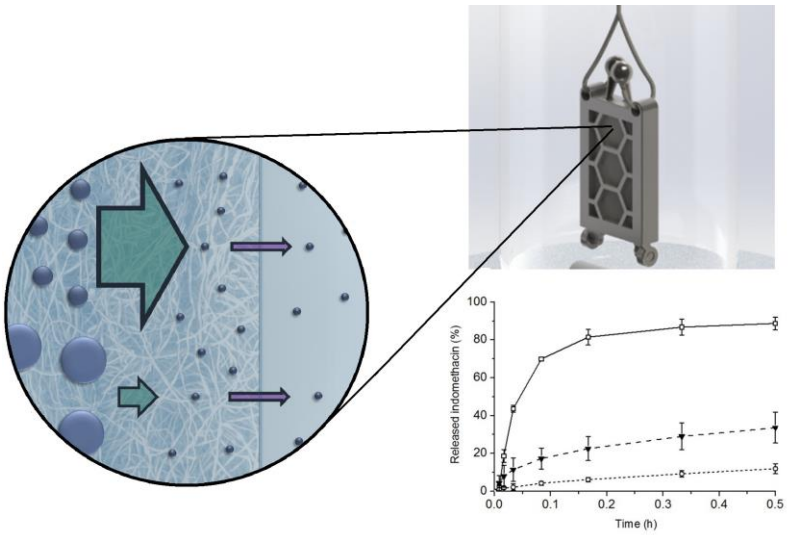
• LM method avoids major issues encountered with current dissolution methods such as the membrane effect and dispersion of the non-dissolved particles.

• LM method permits rapid contact with the dissolution medium, enables separating the dissolved species from the non-dissolved particles, and clearly displays the different dissolution rate of different size particles.

Abstract

We introduce a system with a lyophilic matrix to aid dissolution studies of powders and particulate systems. This lyophilic matrix method (LM method) is based on the ability to discriminate between non-dissolved particles and the dissolved species. In LM method the test substance is embedded in a thin lyophilic core-shell matrix. This permits rapid contact with the dissolution medium while minimizing dispersion of non-dissolved particles without presenting a substantial membrane effect. The method produces realistic dissolution and release results for particulate systems, especially those featuring nanoscale particles. By minimizing method-induced effects on the dissolution profile of nanopowders, the LM method can overcomes shortcomings associated with current dissolution test methods.

Graphical abstract



Keywords:

Dissolution; Dissolution rate; Drug release; Particulate systems; Nanoparticles; Lyophilic matrix method

1. Introduction

The dissolution rate of a drug is a physico-chemical property to be determined and modified during drug discovery and development [1, 2]. For example, reducing the particle size to the nanoscale increases the dissolution rate and thus the bioavailability of poorly water-soluble drugs in classes II and IV of the Biopharmaceutics Classification System [3-5]. The dissolution rate of nanoscale particles correlate with the performance and quality of a formulation featuring nanoparticles [3]. Hence to assess the impact of nanonizing a poorly water-soluble drug, one needs reliable dissolution rate data of nanoparticulate systems. Such data could allow one to predict realistic *in vitro - in vivo* (IVIV)-correlation and facilitate determination of dose in animal experiments [6-8].

Current methods for investigating dissolution rates of nanoscale particles include the United States Pharmacopoeia (USP) I (basket), II (paddle), and IV (flow-through) methods, as well as modifications thereof, membrane diffusion methods (such as the dialysis methods), and sample and separate methods (such as centrifugal ultrafiltration) [6, 7, 9-14]. Additionally, dissolution rates of nanoparticles have been determined from tablets and admixtures using gel matrices [15, 16].Often, the measured values reflect features of the dissolution test device, equipment or method, rather than the nanoparticle properties.

The main issues with the current methods include: dispersion of non-dissolved particles, hydrodynamics-induced variability, membrane effects caused by diffusion barriers (e.g. gelatin, filters, or dialysis membranes), clogging and breaking of filters, sensitivity to flow and location in the dissolution vessel, as well as migration of nanoparticles to interfaces (e.g. wetting issues, floating, or adhesion) [17-24]. The UPS methods were not designed for dissolution studies of nanoscale particles and thus produce unrealistic results [13, 17]. Dispersion and the consequent overestimation of nanoparticle dissolution rates in the USP I and II methods occur when the location of the particles is not fixed. In the USP IV method dispersion occurs when a too large filter pore size is used [6]. On the other hand, constraining diffusion of the dissolved species by membranes or encapsulation, leads to measurement of the quality of the diffusion barrier rather than the nanoparticle dissolution, and often to underestimating the dissolution rate [17, 19, 25, 26]. Using tablets or admixtures may alter the physical form of the drug during the tableting or mixing processes, and they may detach particles from the tablet surface during the dissolution process, or induces a diffusional barrier [15, 16]. Accordingly, there is a need for new methods and devices for determining dissolution rates of nanoparticles.

2. Materials and methods

2.1. Chemicals

Indomethacin (Hawkins, USA) was used as poorly water-soluble model compound in the dissolution experiments and poloxamer 188 (BASF Co., Germany) was used as stabilizer. The chemicals used for preparing the media for the dissolution studies were monopotassium phosphate (Riedel-de Haën, Germany), sodium phosphate dibasic (Sigma-Aldrich, USA), and 5M sodium hydroxide (VWR Chemicals BDH Prolabo, EC). All chemicals employed in the experiments were of analytical grade and used as received.

2.2. Structure of the device

The device used in the dissolution experiments comprised a lyophilic matrix, a cage, a vessel, and a mixing/heating plate (**Fig. 1**). The matrix has a core-shell structure comprising a core matrix, that contained the particles of the test substance, and a surrounding shell matrix. The matrix material of both core and shell matrices is cotton (100% cotton, Curatex GmbH, Germany). The shell matrix consists of four layers of water jet-pressurized cotton with a dry specific surface weight of 5 ± 0.2 mg/cm^2. Cotton was selected as matrix material due to its unique properties; hollow cellulose fibers, high wet strength, inert nature, and substantial ability to absorb water-based media. The custom

designed stainless steel cages (depth 3 mm x height 26 mm x width 16 mm) were 3D printed with selective laser sintering (Mlab Cusing, Concept Labs, Germany). The cage maintained the desired matrix geometry and provided a fixed diffusion distance.

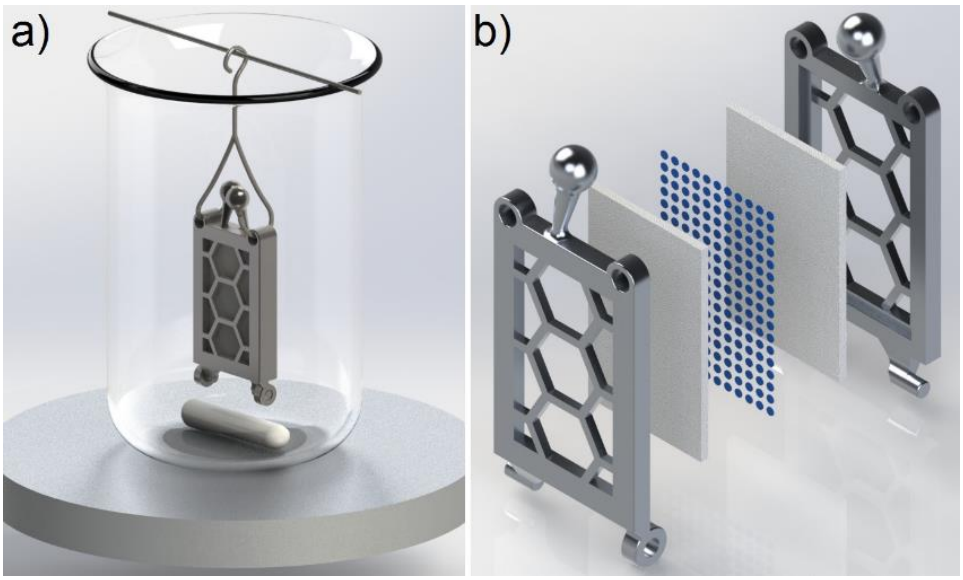


Fig. 1. a) LM method test setup and b) core-shell structure within the LM device, blue dots represent the core matrix containing the particles surrounded on all sides by the shell matrix and cage.

2.3. Characterization of the matrix

2.3.1. Matrix-medium interaction

The cotton matrix was examined prior to, during, and after medium exposure with light microscopy (Leica DMLB, Leica Microsystems Wetzlar, Germany) with a magnification of 200 x, and prior to, and after medium exposure with scanning electron microscopy (SEM, Quanta™ 250 FEG, FEI Inc., USA) with a magnification of 500 x, voltage of 5.00 kV, spot size of 3.0, sputter coated with a 5-nm-thick platinum layer (Q150T Quomm, Beijing, China). The water up-take properties of the matrix were investigated with a fast camera (1200 fps, Casio Exilim High-speed EX-FI1, Casio, Japan) and by weighing the matrix prior to and after exposure to the medium.

2.3.2. Drug-matrixn interaction

The partitioning of the model compound between the matrix and medium was examined by partition coefficient and inverse partitioning coefficient studies. First, the retention of the model compound within the matrix was examined. This was done by partition coefficient tests, where the matrix containing 1 mg of bulk indomethacin was immersed in the medium, and collected after 22 hours.

The indomethacin retained in the matrix was determined by immersing the matrix into fresh medium for 22 hours. This procedure was conducted with three parallel experiments in pH 5.5 and pH 7.4 phosphate buffer media [27] at 37.0 ± 0.5 °C with a stirring rate of 180 rpm (IKA RT 15 P, IKA Werke GmbH & CO. KG, Germany). The concentration of the medium was determined after first and second immersion at time point of 22 h. The concentration of the samples was analyzed with high performance liquid chromatography (HPLC Thermo System Products, Agilent 1200 Infinity Series, Agilent Technologies, Germany), using a Discovery C18 column (4.6 × 150 mm, 5 µm, Supelco, USA), 1.5 mL/min flow rate with a mobile phase consisting of 60:40 (V/V) acetonitrile (ACN) and 0.2% ortophosphoric acid (H_3PO_4) in water (MilliQ), operating at 30 °C with detection at 270 nm. The standard curve for indomethacin quantification was acquired from triplicate samples of indomethacin concentrations between 0.08 mg/L and 500 mg/L ($R^2 = 0.999$).

Second, the partitioning of the dissolved species into the matrix was examined. This was done by inverse partition coefficient tests, where an empty matrix was inserted into medium with saturated concentration of the model compound. Test was conducted in triplicate in phosphate buffer media with pH of 5.5 and 7.4. The empty matrices were inserted into the medium every 5 minutes and the test run was 20 minutes. The concentration of the medium was monitored online using in-situ fiber-optic UV monitoring (Opt-Diss 410, Distek, Inc., USA) using probes with a path-length of 5 mm, exposure time of 44 ms (4 scans/data point) at an analytical wavelength of 320 nm.

2.4. Drug release studies

2.4.1. Preparation and characterization of the particles

A nanosized fraction, two sieved particle size fractions, and bulk indomethachin were tested with the LM method. Nanosuspension was prepared by milling with a Fritsch Pulverisette 7 Premium ball mill (Fritsch GmbH, Germany) to obtain particles for the experiments. Nanoparticles for the LM method were prepared of 2 g indomethacin suspended in solution containing 5.0 mL 0.24 g/mL poloxamer 188 solution (60 wt% relative to the drug amount) and 5.0 mL water (milliQ), and by grinding at 850 rpm in 5 cycles of 3 min using 60 g milling pearls (zirconium oxide, diameter 1 mm). The particle size distribution in the nanosuspension was determined with a Zetasizer Nano SZ (Malvern Instruments Ltd., UK).

The bulk indomethacin was divided into two fractions using a sieve with an eye size of 125 µm (Fritsch GmbH, Germany). The particle size of the bulk powder and the two fractions were determined from SEM images (see section 2.3.1.) (n = 300, ImageJ freeware, National Institutes of

Health, USA). The bulk powder and the two fractions were each mixed with poloxamer 188 (60 wt% relative to the drug amount) to achieve physical mixtures with components identical to the nanosuspension.

2.4.2. Dissolution experiments

The test substances (corresponding to 400 µm of indomethacin) were distributed within the core matrix, the nanosuspension was distributed wet and left to dry. The core matrix was then placed in the matrix holder. Dissolution tests were conducted in triplicate for nanoparticles, bulk powder, and the two particle size fractions in pH 5.5 phosphate buffer medium [27] and for nanoparticles and bulk powder in pH 7.4 phosphate buffer medium [27]. All tests were performed under sink conditions in 100 mL of dissolution medium at 37.0 ± 0.5 °C using a stirring rate of 180 rpm (IKA RT 15 P, IKA Werke GmbH & CO. KG, Germany). The stirring rate and the geometry of the matrix were optimized with preliminary experiments. Aliquots of 1 mL, subsequently replaced with the same volume of fresh medium, were taken at 12 time points: 30 s, 1 min, 2 min, 5 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, and 22 h. The samples were analyzed with HPLC as described in section 2.3.2. Cumulative release of indomethacin and standard deviation in three parallel samples were determined for each experiment.

3. Results and discussion

3.1. Properties of the matrix

3.1.1. Matrix-medium interaction

No visual changes were detected in the size, topology, and morphology of the cotton fibers as the matrix was exposed to dissolution medium, nor did the structure of the cotton change after drying (**Fig. 2**). When immersed into medium, the cotton matrix was wetted in 0.31 s ± 0.10 s. The matrix withdrew medium approximately 23 times its weight. The result of these two experiments indicate that the particles within the matrix are exposed to the medium immediately after immersion. The volume of the matrix increases when exposed to medium. However, microscope studies indicated that the single fibers do not swell when immersed into the medium.

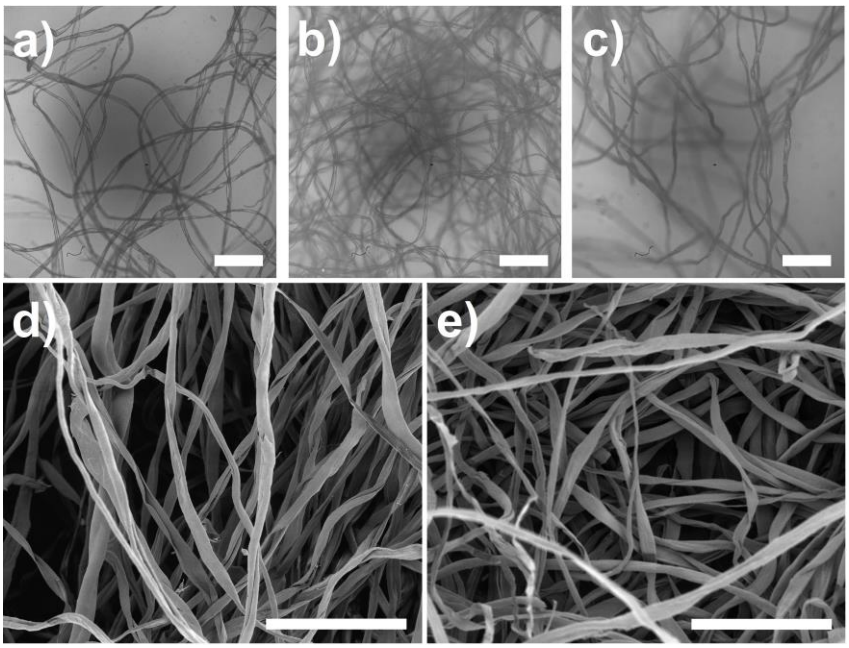


Fig. 2. Light microscope images of the cotton shell matrix a) prior to dissolution medium exposure, b) exposed to medium, and c) after medium exposure. SEM images of the cotton d) prior to medium exposure, and e) after medium exposure. Scale bars correspond to 200 µm.

3.1.2. Drug-matrix interaction

No retained indomethacin was found in the matrix after first 22 hours of in the partition coefficient tests. The concentrations obtained were below the detection limit (0.08 mg/L) of the HPLC method. This indicates that > 99.2 % of indomethacin is released from the matrix and the dissolved species is not significantly retained within the matrix. The inverse partition coefficient test showed no detectable (detection limit: ____) change in concentration when the matrix was immersed into the medium with dissolved indomethacin. This indicates that the matrix does not absorb dissolved indomethacin from the dissolution medium.

As shown in the characterization tests, the matrix is practically inert and has little effect on the total quantity of indomethacin released (**Table 1**). As the pH has no effect on the partition coefficient, we conclude that at least with indomethacin - a weak acid (pK_a 4.5) - the change in the pH of the medium causes no adsorption onto the fibers. The partitioning coefficient studies were conducted only in regard to the dissolved species. The possible adhesion is not considered to be an issue, since the non-dissolved particles are intended to remain within the matrix.

177 **Table 1**

178 Summary of the investigated properties of the matrix.

Property	Experiment	Result
Intake of medium	Weighing	23 x weight of the matrix
Wetting time	Fast camera tests	0.31 ± 0.10 s
Impact of medium on morphology	Imaging	no impact
Adsorption of dissolved species to matrix (from particles)	Partition coefficient	> 99.2 %
Adsorption of dissolved species to matrix (from media)	Inverse partition coefficient	< ___ %

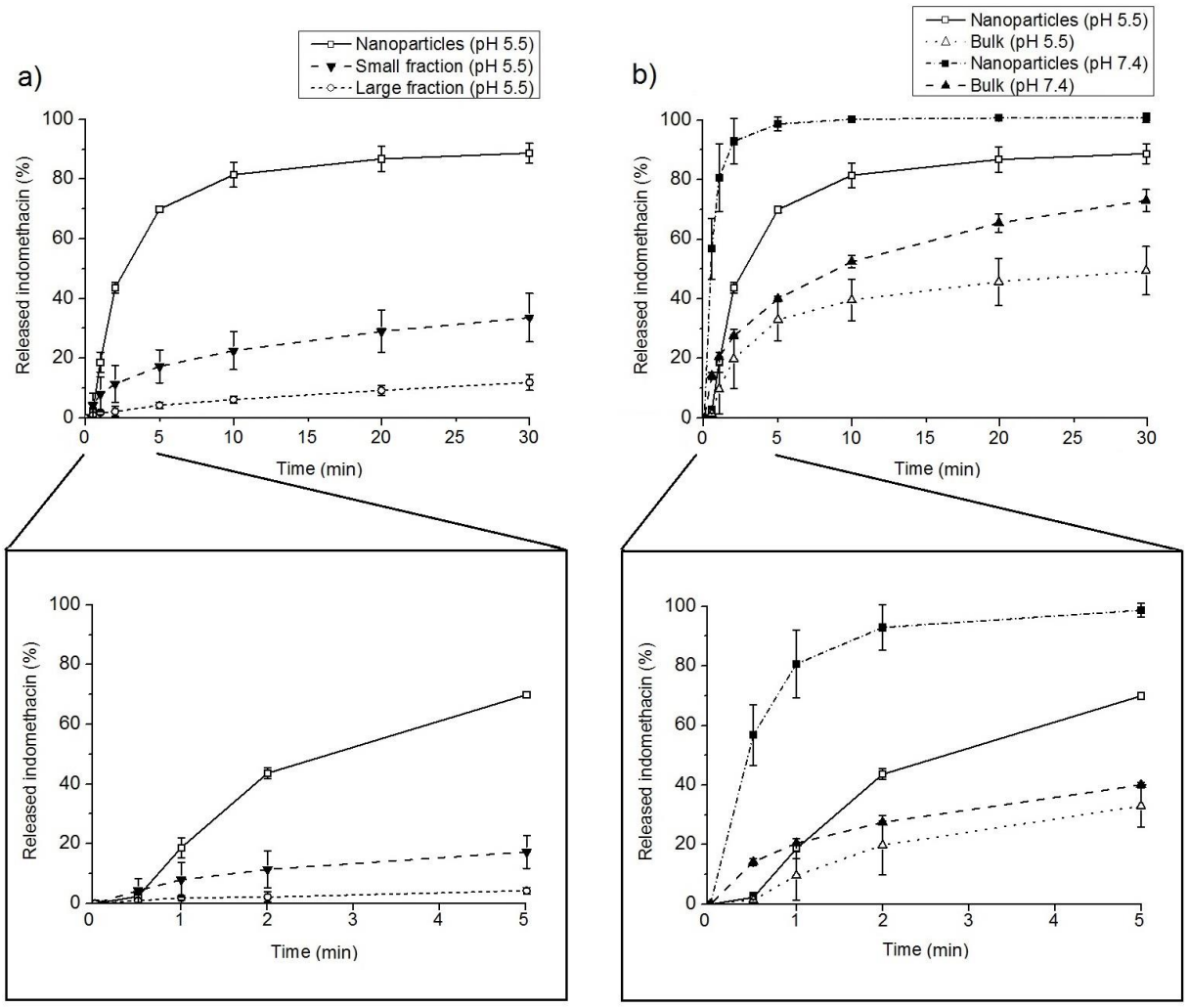
179 *3.2. Drug release studies*

180 *3.2.1. Particle size*

181 The average size of the nanoparticles was 424 nm ± 236 nm, and of the bulk powder 20.3 µm ± 30.0
182 µm, featuring size range of 1 µm - 272 µm. The average size of the small fraction was 17.4 µm ±
183 11.6 µm, and of the large fraction 22.1 µm ± 21.8 µm.

184 *3.2.2. Dissolution rate*

185 Differences in dissolution rate as function of particle size and pH were evident in the dissolution
186 profiles obtained with LM method. **Fig. 3** shows the cumulative release of indomethacin nanoparticles,
187 small and large size fraction in pH 5.5, and nanoparticles and bulk indomethacin in pH 7.4 up to 30
188 min. The short lag times indicated rapid wetting of the samples and absence of any significant
189 membrane effect. Monotonously increasing dissolution profiles and constant standard deviations
190 indicate that the variation between aliquots is moderate, i.e. that no substantial withdrawal of particles
191 occurred during sampling. The method was accurate with small sample quantities and differences
192 between the dissolution rates were detected within 5 minutes from the start of the experiment as seen
193 in the dissolution profiles.



194

195 **Fig. 3.** Cumulative release (%) of indomethacin a) nanoparticles, small fractions and large fraction in
196 pH 5.5 up to 30 min and 5 min at 37.0 ± 0.5 °C, b) nanoparticles and bulk in pH 5.5 and 7.4 up to 30
197 min and 5 min at 37.0 ± 0.5 °C. Error bars are standard deviations of three parallel measurements.

198 *3.3. Principle of the LM method*

199 The key factor of the LM method is its ability to separate non-dissolved particles from dissolved
200 species and its ability to prevent dispersion of the particulates without presenting a significant
201 membrane effect. The dissolved species exit the matrix, whereas the non-dissolved particles remain
202 within the matrix (**Fig. 4**).

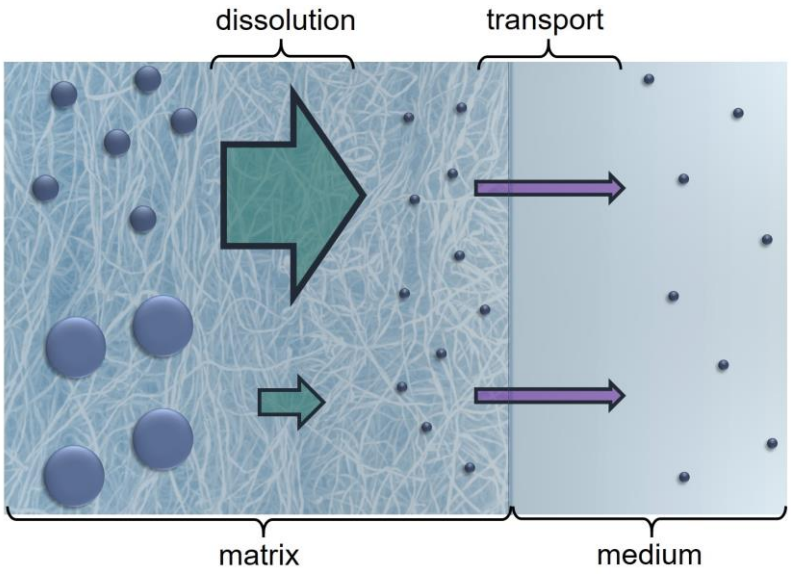


Fig. 4. Dissolved species (small spheres) diffuse promptly into the medium, whereas the diffusion velocity of the non-dissolved species (medium size and large spheres) is lower. Smaller particles (medium size spheres) dissolve to form the dissolved species faster than larger particles (large spheres).

Instead of dispersing the particles into the dissolution medium or exchanging the medium through a barrier, the matrix fixes the position of the non-dissolved particles and brings the medium to the particles. In the LM method the particles are dissolved from a stationary point in a semi 2-dimensional system under sink conditions. The efficient intake of medium, the concentration gradient, and the mild convection induced in the vessel drive the dissolved species out of the matrix. The matrix does not a form a separate compartment in the dissolution vessel and the lack of interaction between the cotton fibers and the model compound ensures that the dissolved species is not trapped in the matrix.

The dissolution of the particles within the matrix is initiated as the matrix absorbs medium. The whole particle population is wetted nearly simultaneously. The dissolution rate depends on the active surface area of the particles as described by the Nernst-Brunner equation and the radius and particle curvature as described by the Gibbs-Kelvin equation [28-30]. The equations predict that small particles dissolve faster than large ones.

Results produced by the LM method do neither overestimate the dissolution rates nor do they induce substantial lag times. The ability to produce realistic dissolution data supports early formulation development, which is valuable for evaluation of advantages gained by particle size reduction and nanonization. Small sample-to-sample variation enables producing reliable results with small inter- or intra-laboratory variation. Thus, it can be inferred that dissolution testing with lyophilic matrices produces realistic estimates of dissolution rates of nanoscale particles. Further studies are needed to

prove the universal applicability of the LM method and to assess the dissolution rate for different substances as well as to verify the IVIV-correlation.

4. Conclusions

The LM method developed in this study is suitable for determining dissolution rates of particulate systems, especially of nanoscale particles. The method features short lag time, small sample-to-sample variation, and monotonously increasing dissolution profiles. It was capable to discriminate the dissolution rates of the tested particle size fractions. The inert cotton matrix used enables release studies without any substantial membrane effect, avoiding dispersion of the non-dissolved particles, and providing rapid wetting of the sample.

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